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ENDOLIMAX REYNOLDSI NOV. SP. FROM THE INTESTINE OF THE COMMON SWIFT, *SCELOPORUS UNDULATUS* *

CLAUDE MATTHEWS MCFALL

During the month of May, 1925, a number of southern lizards were collected in South Carolina and Georgia and their blood and intestinal contents were examined for parasites. This collection included the common swift, sand streaker, glass lizard and chameleon. Parasites were not found in the blood of any specimen examined. On the other hand the intestines of these species were heavily parasitized by various types, such as amoebae, flagellates, coccidia and worms, that afford excellent and abundant material for the student of endoparasitology.

For my own investigations *Sceloporus undulatus* was chosen for two reasons, viz., it is the most numerous of the above named species and it will survive longest in captivity without food. The intestinal contents of more than 80 of the common swifts have been examined. These came from Virginia, the Carolinas and Georgia, but the majority were obtained from South Carolina. All except five very young ones were infected with some of the four forms of parasites previously mentioned. The males have a higher infection than the females, especially during the laying season. Just why this is true, I have not been able to determine. But the very young lizards are not infected.

So far as I have been able to learn no extensive study of the intestinal fauna of the American lizard has ever been made. In Europe, Grassi, Dobell, Hartmann, Prowazek and others have furnished interesting reports on the protozoa found in some lizards in Italy and Germany. *Amoeba lacertae* was discovered by Hartmann and Prowazek in 1907 and later studied by Nägler in 1909. On account of the latter's extreme brevity and confusion in his drawings, Nägler's report leaves much to be desired.

Dobell, in 1914, near Naples, studied an amoeba from the gut of *Lacerta muralis*. His report, while differing materially from Nägler's, leaves one persuaded that they were dealing with the same species. In addition to *Amoeba lacertae,* Dobell says there is often present another form that appears to be a true parasite, an Entamoeba; but as he could not, at that time, find a single dividing form of this species, he decided not to give it a specific name. In describing the motile form he says: "Large specimens possess, when rounded, a diameter of 30μ or slightly more; very small individuals do not exceed 10μ. In the largest forms

* From the Biological Laboratory, University of Virginia.

the diameter of the nucleus is about 6.5μ, in the smallest about 3.5μ. The nucleus contains a large karyosome, surrounded by numerous granules or peripheral chromatin supported on a linin network (Fig. 15). There is a very thin nuclear membrane present. The cytoplasm has a delicate alveolate structure in fixed specimens, and is always packed with food bodies. No contractile vacuole is present. When alive, this organism has an appearance (the nucleus excepted) very much like that of *Entamoeba ranarum,* and moves in a similar manner." Nägler also mentions another amoeba which he calls *Basidiobolus lacertae,* and which he says always accompanies *Amoeba lacertae.* I find something that has an appearance like the amoeboid form to which Nägler gave the generic name of Basidiobolus, but as I have not been able to see the motile form nor its complete stages of division, I should hesitate to call it an amoeba.

Examination for the living forms was made first by taking fecal matter from the cecum. In order to save time it is best to snip off the lizard's head with a pair of sharp curved scissors. The highest infection in starved individuals is always in the region of the cecum. The fecal matter was placed on a slide in a 0.7 per cent normal salt solution and if the organisms were present, they could be seen readily with the 16 mm. objective.

Smears were made from all parts of the large and small intestine; no egg albumin was used unless the fecal matter was insufficient to hold the organisms on to the slide. Various fixatives were used in studying this material, viz., Schaudinn's aceto-sublimate and Bouin's solution. The slides were mordanted for at least two hours, sometimes overnight. They were always left in the stain overnight and sometimes longer. Naturally it takes longer for the destaining process but one gets a much finer definition in the organisms so treated. All smears were made directly from the intestine; although one culture lived for six days in beef extract and a 0.7 per cent normal salt solution, there were never enough of the organisms in this culture to make a successful smear.

The smear method is satisfactory for the study of the flagellates, but for the amoebae fixing the entire intestine in Bouin's solution for several (16 to 18) hours proved much more desirable. This fixation was followed by Heidenhain's iron hematoxylin method of staining, after the tissue is sectioned in paraffin. The amoebae in these preparations are much more numerous because they are less likely to be washed off the slide as they so often are in the smear method. They also stain and destain to a much more desirable degree. More than a hundred slides were prepared by the smear method and the amoebae were found on 90 per cent of them, but always in very small numbers, never more than one or two in each field whereas in the sections, as high as 21 have been counted in one field under the 16 mm. objective.

THE MOTILE AMOEBA

The motile amoeba exhibits two widely different forms; the first (Fig. 1) is characterized by having an extraordinarily long, narrow pseudopodium, a diamond-shaped nuclear membrane, enclosing a thin layer of "peripheral chromatin." It is highly vacuolated but careful observation shows no food vacuoles with food inclusions. The second form is characterized by having broad, blunt pseudopodia (Fig. 11) and cytoplasm highly speckled with faintly staining bodies that cannot be regarded as food, for there are no food vacuoles present. These bodies may possibly be parasitic in nature for the nucleus in figure 11 appears to be undergoing reduction and in figure 12 it has disappeared entirely.

The average diameter, when rounded is 14.5μ. Others measured 13.2 by 20.8μ and one 15 by 45μ (Fig. 1). The smallest specimens averaged about 7.5μ. The diameter of the entire nucleus averages 4.5μ, varying from 3 to 6μ in diameter. Its structure may be considered under four heads, viz. (1) the nuclear membrane; (2) the karyosome; (3) the peripheral layer of granules, and (4) the linin threads.

The nuclear membrane is clearly visible unless the organism is filled with the problematic bodies seen in Figure 12. Where there are none of these bodies (Fig. 1) the nucleus is plainly visible. In most cases, with iron hematoxylin, a border of very fine granules can be seen just inside the nuclear membrane (Fig. 3). These may be chromatin granules but they are not as large as those characteristic of Endamoeba. The membrane is not always spherical, it is very often polyhedral in shape (Fig. 2). The karyosome is centrally located in the motile form, averaging 2.2μ in diameter and varying from 1.5μ in some forms to 3μ in others. In 2,000 measurements, the nucleus was, on the average, one third the diameter of the organism and the karyosome one half the diameter of the nucleus.

In all stains and especially in iron hematoxylin the karyosome is homogeneous; no centriole, as seen by Nägler in *A. lacertae* (Hartmann) has been observed. Even when the stain has been almost completely extracted from the cytoplasm, the karyosome still remains very dark and never appears either alveolar or vacuolated. In some motile forms the zone surrounding the karyosome is not always clear; in some it is more contracted than in others (Fig. 1) and in such cases the linin threads connecting the karyosome with the nuclear membrane are seen with extreme difficulty.

THE CHROMATINIC GRANULES

There will be noticed a small, black granule (Fig. 3) located near the nuclear membrane. Of the 81 motile forms measured, this cytoplasmic feature was not visible in 13 of them, due to two possible

reasons: (1) to the presence of a large number of metaplasmic inclusions (Fig. 12), or (2) to the cytoplasmic area containing this granule having been cut off in sectioning the intestine. This peculiar structure was observed not only in the motile form but was constantly found in the precystic stage also (Fig. 3). In 889 of these precystic stages only 15 were without this chromatinic granule. At first one may confuse it with the other darkly staining bodies shown in figure 12, but by careful focusing it is seen to have a slightly higher refractive index than the other bodies and is also larger. This granule evidently undergoes division into two parts (Fig. 4); then into four parts (Fig. 5). This process is kept up until the granules become scattered throughout the cytoplasm (Fig. 6). The exact nature of this granule has not been determined, and contrary to Dobell I do not find that it is developed at the expense of the karyosome. At times when food is abundant this deeply staining cytoplasmic body is not to be seen. On the other hand, during periods of starvation it is conspicuous. And with the approach of encystment it divides and becomes a prominent feature of the cell about to become a cyst. These facts suggest that this chromatinic, cytoplasmic inclusion may play a nutritional rôle in tiding the organism through the viscissitudes of starvation and propagation. One may say, therefore, that it represents Meltzer's so-called, "factor of safety."

THE PRECYSTIC FORM

The measurements of 889 of this form show that they are, on the whole, slightly smaller than the motile form; averaging 13.1μ in diameter and ranging in size from 7.5 to 22μ in diameter. They are, in most cases spherical in form so that their measurements are quite easily obtained. The diameter of the nucleus and karyosome is only a trifle smaller than in the motile forms; but this diminution is not sufficient to permit one to decide that the chromatinic granule is formed at the expense of the karyosome. The nucleus averages 4.3μ and the karyosome 2.2μ in diameter, and like the motile form, one finds the nucleus and karyosome increasing or diminishing in size with the individual specimen. The linin threads and "peripheral chromatin" are plainly visible in the precystic stage and especially in the binucleate form (Fig. 13) only four of which were seen in the 2,000 specimens measured. These precysts, like the motile forms are seen all the way from the upper jejunum down to the cloaca, exclusive. No invasion of the tissues by the amoeba in any of its stages has been observed.

THE CYST

This amoeba encysts while still in the small intestine and like the other two forms mentioned above, the cyst is found high up in the jejunum and measurements of 1,030 of them give an average diameter of

12.4μ. The nucleus averages 4.3μ in diameter, while the karyosome in these has an average of 2.2μ. The cysts vary from 7.5 to 22μ in diameter. When compared with both the motile and precystic forms it will be seen that the organism undergoes a reduction of only 2μ in diameter before complete encystment is accomplished. The measurements also show that there is very little variation in the size of the karyosome previous to encystment; the structural diminution seems to be confined chiefly to the cytoplasm and nuclear membrane.

When examined in the living state the cytoplasm appears quite granular and the nucleus very indistinct. The cysts do not react to iodine. In prepared specimens the cyst takes a very deep stain, blue to almost black, but destains more readily than either the precyst or motile form. The karyosome is indistinct (Fig. 9). Previous to encystment all food is voided, the deeply staining chromatinic granules collect near the periphery and the cytoplasm begins to draw away from the cyst wall. This wall is composed of two layers, an outer dark brown layer and an inner yellowish layer (Figs. 7, 8, 9).

No suggestion of a nuclear division has been observed in either the so-called precyst or cyst proper. Only a few of the 81 motile forms studied showed any disposition to divide, so that a complete cycle cannot be given at present. The only suggestion of division observed in the motile form was seen in a case where the chromatin of the karyosome had divided into four granules, two large and two small (Fig. 10); and a second case which was binucleate (Fig. 13).

There was no suggestion of spindle formation in any of these stages such as has been described by Nägler and none was observed by Dobell in the specimens that he studied. It seems very remarkable that in over 100 slides made from fecal smears, and in sectioned intestines of 80 lizards a complete nuclear division was not seen. It may be, however, that division takes place very rapidly in these forms and that one would come upon the division stages by chance. Lack of material prevents further investigation just now.

DISCUSSION

Dobell points out in his report on *A. lacertae,* that there are two amoebae commonly found in the hindgut of *Lacerta muralis;* an Endamoeba (Fig. 15), evidently a parasitic type, and *A. lacertae* (Fig. 14) which is usually accompanied by *Basidiobolus lacertae.* No Endamoeba as illustrated by Dobell has been observed in any of the species of American lizards that have been studied. The basis for assuming that Dobell and Nägler have been dealing with the same species rests upon the nuclear division and not upon the size of the organism. Nägler's report has been so carefully reviewed by Dobell in his own report that it would be a waste of space for me to go over the argument again. The question

now is: Have I been dealing with the same amoeba in *Sceloporus undulatus* that Dobell found in *Lacerta muralis?* In but one respect does it resemble Dobell's amoeba. It resembles his Endamoeba in that division stages could not be definitely established. In seven months of almost continuous investigation only two indefinitely dividing forms, mentioned before, have been observed. Dobell says he worked to the point of exhaustion on his Endamoeba and did not find a single dividing form and for that reason has hesitated to give it a specific name. Our greatest divergence is in the size of the motile form. Dobell's varies from 7 to 14μ in diameter, while mine has a variation of 10.5 to 21μ in diameter. The nucleus of my amoeba varies from 3 to 6μ, his from 3 to 4.5μ. He does not give the size of the karyosome in the motile form but states that it is relatively large. The karyosome in mine varies from 1.5 to 3μ. We agree in that it is homogeneous in character and filled with deeply staining granules; but I have not been able to find them imbedded in a plastic matrix as he has, and in no case does the karyosome ever appear vacuolated.

Dobell finds no "peripheral chromatin" in the clear zone about the karyosome and no linin threads connecting the karyosome with the nuclear membrane. I find a peripheral cloud inside the nuclear membrane and linin threads connecting the karyosome with the nuclear membrane in some specimens. These features are seen only with great difficulty in some specimens and not at all in others. We agree that dividing forms are rounded, but I have seen no pseudopodia and nothing like spindle formation in these forms. In the encystment of the organism we are more nearly agreed than in the comparison made above; Dobell, however, does not describe any transitory stage such as the precystic stage.

A. lacertae, Dobell states, encysts in the hindgut of the lizard. In my investigations cysts and precysts were found high up in the jejunum and ileum and from there on down to the cloaca. I have never seen either a precyst or motile form from the cloaca in *Sceloporus undulatus.* In 889 precysts and 1,030 cysts measured, very little change in the diameter of the karyosome takes place; it is, on the whole, one-half the diameter of the nucleus. As Dobell observed in the cysts of *A. lacertae,* the amoeba dealt with in this paper, gets rid of all food in the precystic stage and becomes spherical; while the linin threads show more plainly in these forms. The origin and purpose of the deeply staining masses, that we both have observed in relation to the nucleus, have not been explained by Dobell. As previously stated, these chromatininc granules apparently originate from a single granule lying near the nuclear membrane, and by successive division become scattered throughout the cytoplasm. It would appear that this substance is used to tide the

organism over the precystic stage and when this transitory period is passed, it is cast out to the periphery of the cytoplasmic wall. About this time the nuclear membrane begins to contract and some of the cysts show some very irregular outlines. I do not find, as Dobell does, that the cysts are hard and impervious to stains; on the contrary, they take such a deep stain and destain so readily that one is able to make out their structure only with great difficulty.

SUMMARY

The amoeba found in the intestine of *Sceloporus undulatus* agrees with *A. lacertae,* as described by Dobell except that:

1. It is larger in the motile, precystic and cystic stages.
2. The nucleus is never vacuolated.
3. A cloud of very fine granules is found inside of the nuclear membrane in motile forms.
4. There is a precystic stage.
5. This organism has a habitat from the upper jejunum down to the cloaca, exclusive.
6. The karyosome averages one half the diameter of the nucleus in the 2,000 forms measured.
7. This organism is characterized in all its precystic stages by a constant chromatinic granule or granules, situated near the nuclear membrane.
8. Division, similar to amitosis, takes place in this granule, until its offspring become scattered throughout the cytoplasm.
9. The cyst stains and destains readily.

From these diverging facts it is evident that this is a new species of amoeba. It apparently belongs to the genus Endolimax; and for the specific name I suggest the name *E. Reynoldsi* as a tribute to the teacher who has been an ever present help and inspiration in my investigations, Dr. Bruce D. Reynolds. I am greatly indebted to Professor William A. Kepner and Dr. James B. Looper for their many courtesies, and particularly to Professor Robert W. Hegner for the perusal of my manuscript.

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Explanation of Plate XXI

The illustrations are drawings and a photograph from sections of the entire small intestine and hindgut of *Scleoporus undulatus* fixed in Bouin's solution and stained with Heidenhain's iron hematoxylin.

Fig. 1.—Motile form showing extended pseudopodium, vacuolated cytoplasm, elongated nucleus and karyosome.

Fig. 2.—Motile form showing polyhedral nucleus and a reticulated cytoplasm.

Fig. 3.—Motile form showing a typical nucleus with central karyosome and linin threads, "peripheral chromatin" and a darkly staining, extranuclear chromatic granule.

Fig. 4.—Early precystic stage showing chromatinic granule dividing.

Fig. 5.—A precystic stage showing chromatinic granule breaking up into four parts.

Fig. 6.—A large precystic stage showing nine chromatinic granules scattered throughout the cytoplasm.

Fig. 7.—Early cystic stage showing light cyst wall and numerous chromatinic granules scattered throughout cyst.

Fig. 8.—Later cystic stage showing chromatinic granules massed around periphery of the cytoplasm.

Fig. 9.—Mature cyst showing chromatinic granules lying at the extreme periphery of cytoplasm.

Fig. 10.—A motile form showing karyosome broken up into four discreet bodies; the chromatinic granule pictured above and many darkly staining bodies of a problematic nature; see text.

Fig. 11.—A motile form showing blunt pseudopodium with a distinction between ecto- and endoplasm; nucleus partially obscured; a single chromatinic granule and many darkly staining bodies.

Fig. 12.—A motile form similar to the last with exception that the darkly staining bodies are more numerous while the nucleus and chromatinic granules are lacking.

Fig. 13.—One of the four binucleate forms encountered. Note the six chromatinic granules three of which are irregular in outline.

Fig. 14.—*A. lacertae* after Dobell, $\times$ 2,000.

Fig. 15.—An "Entamoeba" found in *Lacerta muralis*, after Dobell, $\times$ 2,000.

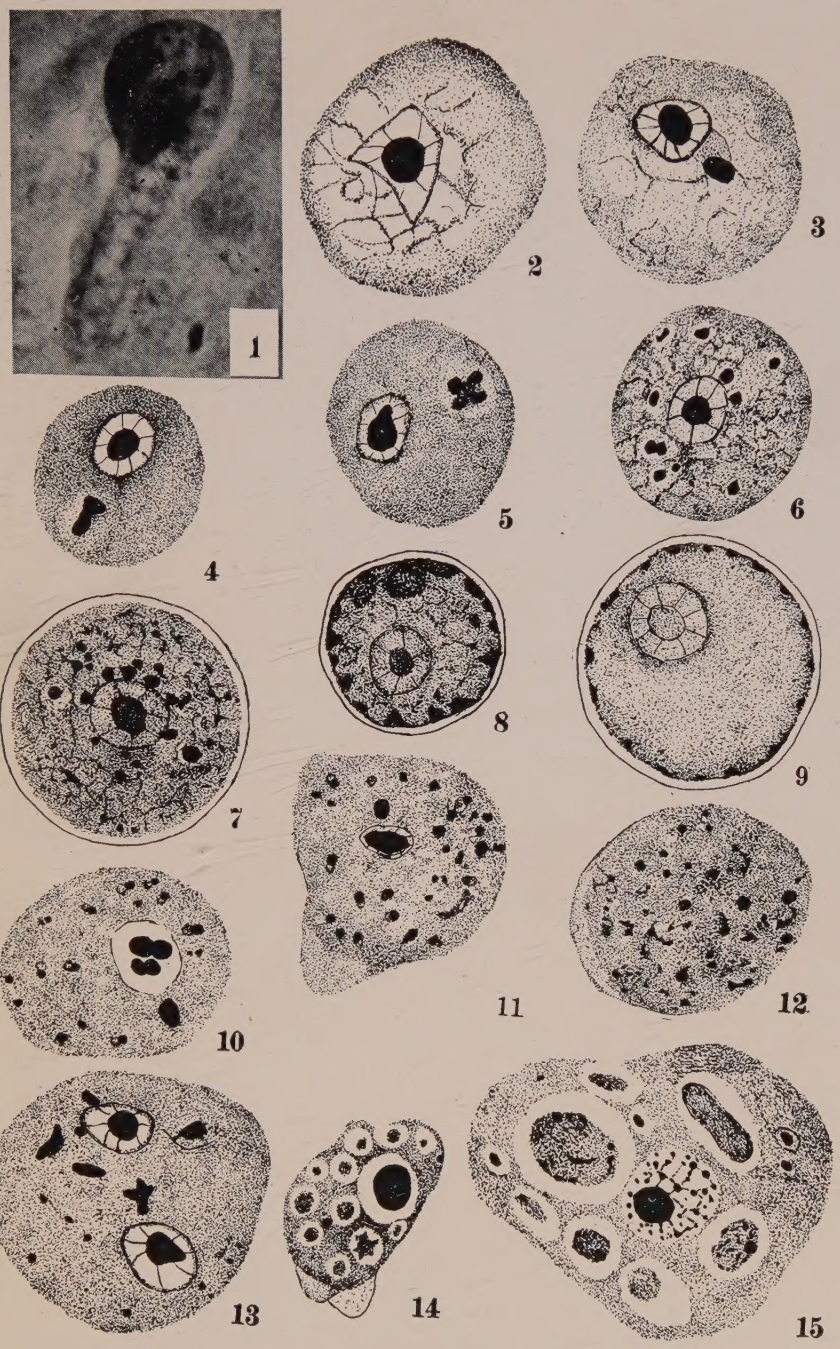

PLATE XXI

THE

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